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High-dose Alkylating Agents with Autologous Hematopoietic Stem Cell Support and Trastuzumab in ERBB2 Overexpressing Metastatic Breast Cancer: A Feasibility Study

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Abstract. *Background: ERBB2 overexpression predicts a worse outcome for patients receiving high-dose chemotherapy (HDC). Trastuzumab improves response rate and survival in ERBB2 overexpressing metastatic breast cancer patients (MBC). We investigated the feasibility of combining high-dose alkylating agents with autologous hematopoietic stem cell (AHSC) support and trastuzumab in ERBB2 overexpressing MBC. Patients and Methods: Eleven consecutive patients with pre-treated ERBB2 overexpressing MBC were enrolled. HDC regimen consisted of a single course of cyclophosphamide 120 mg/kg + melphalan 140 mg/m^2 (CyMEL, n=8), a single course of Thiotepa 600 mg/m^2 (TTP, n=1) or a sequential combination of Thiotepa 600 mg/m^2 followed on day 21 by BCNU 600 mg/m^2 (TTP-BCNU, n=2). Trastuzumab (4mg/kg) was started 24 h after AHSC infusion and then administered weekly (2 mg/kg). Results: Median time to neutrophil and platelet recovery was 10 and 14.5 days, respectively. Three patients experienced febrile neutropenia and in 2 Herpes virus infections were documented. Five grade III/IV mucositis/oesophagitis were recorded. One patient experienced a reversible atrial arrhythmia on day 2 of trastuzumab, and another patients had a nonsymptomatic decrease in LVEF >10% on week 12 of trastuzumab. No toxic death was recorded. Median time to progression was 5 months (1 to 38 +). Conclusion: Combining alkylating agent-based HDC and trastuzumab appears to be feasible in ERBB2 overexpressing MBC and warrants further investigation in a larger cohort.*

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High-dose chemotherapy (HDC) with autologous hematopoietic stem cell (AHSC) support remains a controversial strategy in breast cancer (1, 2). In spite of various promising early phase II trials suggesting substantial improvement of outcome, no randomised, controlled studies demonstrated any overall survival benefit over full-dose conventional chemotherapy (3-11), though some authors reported trends or significant advantages in event-free survival. Nevertheless, subset analysis of some of these trials suggest that the impact of HDC might differ according to the biological phenotype of tumours, the molecular characteristics of which remain to be clearly defined (12-17). Among potential candidates, ERBB2 oncoprotein could play a critical role. Hence, overexpression of ERBB2 that results from amplification of the *ERBB2* gene in 20 to 25% of all primary breast cancer is classically associated with a poor clinical outcome (18, 19). Importantly, ERBB2 overexpression has been identified by several groups, including ours, as a major adverse prognostic factor in breast cancer patients receiving alkylating agent-based HDC (12, 15, 17, 20, 21), suggesting that HDC is not able to overcome the adverse effect of ERBB2 on outcome in this disease.

When associated with conventional chemotherapy, trastuzumab (Herceptin®) –a recombinant humanized monoclonal antibody targeting ERBB2– was demonstrated to drastically improve the response rate as well as the progression-free survival and the overall survival of ERBB2 overexpressing MBC patients (22). In addition, preclinical evidence supports the existence of a synergism between alkylating agents and trastuzumab in human breast cancer models (23). Consequently, it can be suggested that strategies combining trastuzumab and HDC might reverse the negative prognostic value of ERBB2 overexpression in breast cancer patients, justifying exploration of the feasibility of such an approach in a clinical study.

Table I. *Patient characteristics.*

	Patient number
	11
Median age	49 years (31-62)
Characteristics of primary tumour	
PTis/pTx	3
pT1/ pT2	6
pT3/pT4	2
pN+	7
M+	1
HR(+/-)	6/5
Previous adjuvant chemotherapy	
Yes/no	7/4
Previous adjuvant radiotherapy	
Yes/no	9/2
Characteristics of metastatic disease	
Median disease-free interval (range)	36 months (0-162)
Visceral involvement	9
Liver	5
Lung	5
Bone and/or soft tissue only	2
Number of metastatic sites	
1	3
2	4
3+	4
Pretreatment for metastatic disease	
First-line	5
Second-line	4
More	2
Chemosensitive/Refractory	6/5

Herein, we report the preliminary results of a pilot study evaluating the toxicity and feasibility of a combination of high-dose alkylating agents administered with AHSC support and trastuzumab in 11 ERBB2 overexpressing metastatic breast cancer patients.

Patients and Methods

Patients. Between January 2001 and April 2002, 11 consecutive patients with metastatic breast cancer were included in this study. To be enrolled, they had to be eligible for HDC according to institutional policy, *i.e.* age under 65 and adequate medical condition, and for trastuzumab treatment, *i.e.* ERBB2 overexpression, left ventricular ejection fraction ≥ 50%, absence of severe pulmonary disease and no previous treatment with trastuzumab. ERBB2 overexpression was determined by immunohistochemistry (IHC) using Herceptest (DAKO), as previously described (20). Only patients with a score of 3+ were included. Other eligibility criteria included adequate bone marrow function, renal function and hepatic function, and written informed consent according to all required guidelines. HDC was delivered either as consolidation of first-line anthracycline -and taxane- based chemotherapy or as salvage treatment in a refractory setting.

Pre-treatment evaluation included complete physical examination with medical history, complete blood count with differential, liver and renal function test, ECG and echographic or isotopic left ventricular ejection fraction and appropriate radiological tumour measurements.

High-dose chemotherapy with AHSC support and trastuzumab treatment. Leukapheresis were performed after a cycle of high-dose cyclophosphamide (3g/m^2) followed by subcutaneous recombinant G-CSF (Filgrastim, Neupogen®, Amgen). All patients had harvested peripheral blood stem cells (CD34+) > 2.10^6 /kg.
Three HDC regimen were used:
- Two patients received a sequential combination of thiotepa 600 mg/m^2 and 21 days later BCNU 600 mg/m^2, both injections followed by AHSC reinfusion
- One patient received only 600 mg/m^2 TTP + AHSC
- Eight patients received cyclophosphamide 60 mg/kg day 1-2 and melphalan 140 mg/m^2 day 3

Each regimen was administered in a conventional hospital unit. AHSC were reinfused 24 hours after HDC and Filgrastim 300 μg/d was subcutaneously administered until ANC exceeded 0.5.10^9 /l for three consecutive days.

Trastuzumab was started 24 hours after stem cell reinfusion at 4 mg/kg as a 90 minutes *i.v.* and then subsequently administered weekly at 2 mg/kg as a 30 minutes *i.v.* Trastuzumab was continued until disease progression or toxicity.

Follow-up and treatment evaluation. Patients were either allowed to be managed on an outpatient basis after high-dose chemotherapy and AHSC reinfusion or discharged of the hospital only after recovery of nonhematologic and hematologic toxicities. All patients receiving at least one injection of trastuzumab were considered assessable for toxicity. Toxicity was assessed using NCI common toxicity criteria (24). Evaluation of LVEF by identical means used for initial evaluation was performed every 8 weeks. Tumour assessments were performed every 8 weeks after the last course, by identical means used for the initial evaluation. Standard response criteria, as defined by the WHO, were used. The time to progression was defined as the time between HDC and the first evidence of disease progression.

Results

Patient characteristics (Table I). Eleven ERBB2 overexpressing metastatic breast cancer patients, not previously treated by trastuzumab, were included in this study. All had received previous cytotoxic treatment for metastatic relapse. Five patients received HDC and trastuzumab as a consolidation after first-line treatment containing taxanes and anthracyclines, for a sensitive disease. The remaining 6 patients received HDC and trastuzumab as part of second-(n=4) or third-line treatments (n=2), 5 of them presenting a disease refractory to a conventional dose of cytotoxics.

All but one had received taxanes for metastatic disease and all patients had been exposed to anthracyclines with a

Table II. *Toxicities.*

	No of patients
HDC regimen	
CyMel	8
TTP/BCNU	2
TTP	1
Hematological toxicity	
Median time to neutrophil recovery (range)	10 days (5-14)
Median time to platelet recovery (range)	14.5 days (11-30)
Erythrocyte transfusion (median number of units)	8 (2)
Platelet transfusion (median number of units)	5 (1)
Febrile neutropenia	8
Documented bacteriemia	3
Viral infection	2
Nonhematological toxicity	
Mucositis/oesophagitis	5
Cardiac events	2
Biochemical hepatic alterations	2
Trastuzumab treatment	
Median number of injections	15
Range	1-38

median cumulative dose of epirubicin or equivalent of 650 mg/m^2. Five patients had liver metastasis, 5 had pulmonary metastasis, 2 patients had bone or soft tissue disease only and 8 patients had at least 2 sites of disease, 4 of them having 3 or more metastatic sites.

The conditioning regimen included either CyMel (n= 8) or a sequential combination of Thiothepa and BCNU with an interval of 21 days (n=2; one additional patient received only Thiotepa due to an insufficient number of hematopoietic stem cells).

Hematological and noncardiac toxicities (Table II). No toxic death was observed. All patients experienced prompt and severe aplasia. The median time to neutrophil and platelet recovery (> 0.5 G/l and > 20 G/l, respectively) from reinfusion of stem cells was 10 (5-14) and 14.5 (11-30) days, respectively. Neutropenia was febrile in 8 patients requiring *i.v.* antibiotics. Bacteriemia was documented in 3 patients. One patient presented a severe gram-negative septicaemia, the outcome of which was quickly favourable with macromolecule and hydration loading and *i.v.* antibiotics. Five patients experienced grade 3 or 4 mucositis and/or oesophagitis. Two patients experienced moderate, nonsymptomatic and reversible alteration of the hepatic biochemical profile (transaminases increase < 5 times the upper limit and total bilirubin < 1.5 times the upper limit).

One patient developed meningitis with high positive Herpes simplex virus antibody (HSV) and delayed positive culture for HSV in the cerebro-spinal fluid, on day 1 after trastuzumab. Treatment required 15 days *i.v.* antiviral treatment followed by 1 month oral treatment. She had just completed severe Herpes Zoster infection before receiving HDC. Another patient presented one month after HDC a severe cervical and thoracic eruption consistent with Varicella-Zoster virus infection, requiring hospitalisation and *i.v.* antiviral treatment for 8 days. Trastuzumab was discontinued for one month. The outcome was quickly favourable and trastuzumab was restarted for a total duration of 6 months, without any recurrence.

Overall, 5 patients transiently discontinued trastuzumab within the 30 days post HDC because of persistent fever. Fever was due to febrile neutropenia or associated to fungal infections in patients with severe mucositis and/or oesophagitis. All patients restarted trastuzumab before day 40.

Cardiac monitoring. No clinically detectable heart failure was observed but 2 significant cardiac events were recorded, one of them being related to trastuzumab treatment.

One patient developed a nonsymptomatic decrease of LVEF from 73 to 53% (*i.e.* a relative decrease of more than 25%) after 13 injections of trastuzumab. She was 50 years old without any history of heart disease and without previous exposure to left chest wall radiotherapy. At the time of metastatic relapse, she had been exposed to 2 cycles of 75 mg/m^2 doxorubicin. Trastuzumab was discontinued and LVEF remained constantly in excess of to 50%.

Another patient developed atrial fibrillation on day 2 after trastuzumab. She was 62 years old with no history of heart disease and had received left chest wall radiation but no adjuvant chemotherapy for treatment of the primary tumour. Metastatic relapse (liver and abdominal lymph node) occurred 48 months later. She received 5 courses of docetaxel (75 mg/m^2) and epirubicin (100 mg/m^2), but had progressive disease at time of HDC. Pretreatment LVEF was 70%. The conditioning regimen was cyclophosphamide-melphalan. Atrial fibrillation was detected on day 2 after trastuzumab and was associated with a concomitant nonsymptomatic moderate decrease of LVEF to 50%. This episode was concomitant with febrile neutropenia and anemia (7 g/dl). No hydro-electrolytic perturbation was observed at the time of arrhythmia. Ventilation/perfusion scintigraphy suggested a limited pulmonary embolism, which was not confirmed by contrast-enhancement, computed tomography. The latter examination revealed a thoracic progression of disease with images consistent with carcinomatous mediastinitis. Atrial fibrillation resolved quickly after *i.v.* amiodarone treatment and erythrocyte transfusion. Trastuzumab was discontinued after the first injection and was not restarted.

Table III. *Antitumour activity.*

Tumour response	No. of patients (%)
OR	7 (64%)
CR	1 (9%)
PR	6 (54%)
SD	2 (18%)
PD	2 (18%)

Response and follow-up. Seven patients experienced an objective response to HDC + trastuzumab, including 1 durable complete response (persistent at the last visit, after 38 months of follow-up) and 6 partial responses, 2 of them being of short duration. Four of those patients were receiving HDC + trastuzumab as part of their first-line treatment.

Two additional patients had a stable disease for less than 6 months and 2 patients had a progressive disease (3 of those patients were refractory to a conventional dose of cytotoxics). All patients have discontinued trastuzumab to date, 7 due to a progressive disease. Among those 7 patients, 2 had a brain-only progression, and trastuzumab was restarted after whole brain radiation. The median time on treatment was 15 weeks (1 to 39) and median time to progression was 5 months (1 to 38 +).

Discussion

The aim of the present pilot study was to investigate the feasibility of combining high-dose alkylating agents with AHSC support and trastuzumab administration in ERBB2 overexpressing MBC. Despite the relatively small number of patients, our results strongly suggest that such a combination is feasible and that trastuzumab does not significantly alter the expected toxicity profile of HDC at the hematological and nonhematological level.

At the hematological level, times to neutrophil and platelet recoveries did not differ from those previously reported following HDC regimen +AHSC support (25, 26). The rate of febrile neutropenia (80%) and documented bacteriemia (30%) were also expected. Only one patient developed a severe bacterial infection, which was rapidly reversible with appropriate management. Surprisingly, 2 Herpes-virus- related events were recorded within the 30 days after HDC, one documented meningitis and one extensive Herpes Zoster infection. Such infectious events have not been described in previous large phase II and III studies of trastuzumab + chemotherapy, but a ecent report describes a rate of nearly 10% of Varicella-Zoster virus reactivation in breast cancer receiving standard HDC and AHSC support (27). Importantly, those 2 patients had persistent severe lymphopenia (<0.5/G/l), and had received docetaxel-based pretreatment with steroids premedication. In addition, Herpes infections occurred relatively soon after trastuzumab initiation (on day 2 and on day 28) and did not reoccur at the time of trastuzumab reinduction. Consequently, viral infections might be more related to previous treatment-related alterations of immune function rather than trastuzumab.

The nonhematological toxicities were those classically expected after high-dose alkylating agents, essentially mucositis and/or oesophagitis. At the cardiac level, no clinically significant adverse event was recorded. One patient experienced transient atrial fibrillation 24 hours after trastuzumab initiation, concomitant with febrile neutropenia and anemia with a simultaneous moderate decrease of LVEF (70 to 50%). Atrial fibrillation was reversible on appropriate treatment and after erythrocyte transfusion. A concomitant progressive carcinomatous mediastinitis was observed. Another patient experienced a nonsymptomatic decrease of LVEF after 13 injections and discontinued treatment. Her LVEF remained higher than 50% and the patient never required a specific treatment.

Of note, and as expected, significant antitumour activity was observed with 7 patients developing an OR, including a persistent long-lasting CR. However, this activity was essentially restricted to chemo-sensitive patients, where HDC and trastuzumab were applied as a consolidation and in a first-line setting, preventing us from drawing any clear conclusions at this level.

To date, no randomised studies have demonstrated a clear survival benefit for HDC + AHSC support over conventional approaches, either in high-risk early primary as well as in metastatic breast cancer (3-11). However, a trend to improvement in progression-free survival has been suggested in certain studies. Moreover, subset analyses of various trials have identified clinical and biological factors that could predict for outcome after HDC (12-17). Among those, we and others have clearly pointed out ERBB2 overexpression as a strong adverse parameter in high-risk early or metastatic breast cancer patients receiving HDC with AHSC support (12, 15, 17, 20, 21).

Altogether, the dramatic impact of trastuzumab on the outcome of ERBB2-positive breast cancer patients in recent randomised trials (22), the preclinically demonstrated synergism between alkylating agents and trastuzumab (23), and the lack of significant alterations of the HDC toxicity profile we observed in our study, make specific ERBB2 targeting and high-dose alkylating agents combination an attractive approach for evaluation in this specific subgroup.

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References

1 Hortobagyi GN: What is the role of high-dose chemotherapy in the era of targeted therapies? J Clin Oncol *22(12)*: 2263-2266, 2004.

2 Nieto Y, Jones RB and Shpall EJ: High-dose chemotherapy for breast cancer: is another look warranted? Curr Opin Oncol *16(2)*: 114-119, 2004.

3 Rodenhuis S, Bontenbal M, Beex LV *et al*: High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer. N Engl J Med *349(1)*: 7-16, 2003.

4 Stadtmauer EA, O'Neill A, Goldstein LJ *et al*: Conventional-dose chemotherapy compared with high-dose chemotherapy plus autologous hematopoietic stem-cell transplantation for metastatic breast cancer. Philadelphia Bone Marrow Transplant Group. N Engl J Med *342(15)*: 1069-1076, 2000.

5 Lotz JP, Cure H, Janvier M *et al*: Intensive chemotherapy and autograft of hematopoietic stem cells in the treatment of metastatic cancer: results of the national protocol Pegase 04. Hematol Cell Ther *41(2)*: 71-74, 1999.

6 Hortobagyi GN, Buzdar AU, Theriault RL *et al*: Randomized trial of high-dose chemotherapy and blood cell autografts for high-risk primary breast carcinoma. J Natl Cancer Inst *92(3)*: 225-233, 2000.

7 Rodenhuis S, Richel DJ, van der Wall E *et al*: Randomised trial of high-dose chemotherapy and haemopoietic progenitor-cell support in operable breast cancer with extensive axillary lymph-node involvement. Lancet *352(9127)*: 515-521, 1998.

8 Roche H, Viens P, Biron P *et al*: High-dose chemotherapy for breast cancer: the French PEGASE experience. Cancer Control *10 (1)*: 42-47, 2003.

9 Tallman MS, Gray R, Robert NJ *et al*: Conventional adjuvant chemotherapy with or without high-dose chemotherapy and autologous stem-cell transplantation in high-risk breast cancer. N Engl J Med *349(1)*: 17-26, 2003.

10 Leonard RC, Lind M, Twelves C *et al*: Conventional adjuvant chemotherapy *versus* single-cycle, autograft-supported, high-dose, late-intensification chemotherapy in high-risk breast cancer patients: a randomized trial. J Natl Cancer Inst *96(14)*: 1076-1083, 2004.

11 Zander AR, Kroger N, Schmoor C *et al*: High-dose chemotherapy with autologous hematopoietic stem-cell support compared with standard-dose chemotherapy in breast cancer patients with 10 or more positive lymph nodes: first results of a randomized trial. J Clin Oncol *22(12)*: 2273-2283, 2004.

12 Nieto Y, Nawaz S, Jones RB *et al*: Prognostic model for relapse after high-dose chemotherapy with autologous stem-cell transplantation for stage IV oligometastatic breast cancer. J Clin Oncol *20(3)*: 707-718, 2002.

13 Rowlings PA, Williams SF, Antman KH *et al*: Factors correlated with progression-free survival after high-dose chemotherapy and hematopoietic stem cell transplantation for metastatic breast cancer. Jama *282(14)*: 1335-1343, 1999.

14 Nieto Y, Cagnoni PJ, Shpall EJ *et al*: A predictive model for relapse in high-risk primary breast cancer patients treated with high-dose chemotherapy and autologous stem-cell transplant. Clin Cancer Res *5(11)*: 3425-3431, 1999.

15 Nieto Y, Cagnoni PJ, Nawaz S *et al*: Evaluation of the predictive value of Her-2/neu overexpression and p53 mutations in high-risk primary breast cancer patients treated with high-dose chemotherapy and autologous stem-cell transplantation. J Clin Oncol *18(10)*: 2070-2080, 2000.

16 Somlo G, Simpson JF, Frankel P *et al*: Predictors of long-term outcome following high-dose chemotherapy in high-risk primary breast cancer. Br J Cancer *87(3)*: 281-288, 2002.

17 Nieto Y, Nawaz S, Shpall EJ *et al*: Long-term analysis and prospective validation of a prognostic model for patients with high-risk primary breast cancer receiving high-dose chemotherapy. Clin Cancer Res *10(8)*: 2609-2617, 2004.

18 Slamon DJ, Clark GM, Wong SG *et al*: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science *235(4785)*: 177-182, 1987.

19 Muss HB, Thor AD, Berry DA *et al*: c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med *330(18)*: 1260-1266, 1994.

20 Braud AC, Mathoulin Portier MP, Bardou VJ *et al*: Overexpression of erb B2 remains a major risk factor in non-metastatic breast cancers treated with high-dose alkylating agents and autologous stem cell transplantation. Bone Marrow Transplant *29(9)*: 753-757, 2002.

21 Guarneri V, Bengala C, Orlandini C *et al*: HER2 overexpression as a prognostic factor in metastatic breast cancer patients treated with high-dose chemotherapy and autologous stem cell support. Bone Marrow Transplant *34(5)*: 413-417, 2004.

22 Slamon DJ, Leyland-Jones B, Shak S *et al*: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med *344(11)*: 783-792, 2001.

23 Pegram M, Hsu S, Lewis G *et al*: Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene *18(13)*: 2241-2251, 1999.

24 Ajani JA, Welch SR, Raber MN *et al*: Comprehensive criteria for assessing therapy-induced toxicity. Cancer Invest *8(2)*: 147-159, 1990.

25 Bertucci F, Viens P, Gravis G *et al*: High-dose chemotherapy with hematopoietic stem cell support in patients with advanced epithelial ovarian cancer: analysis of 67 patients treated in a single institution. Anticancer Res *19(2B)*: 1455-1461, 1999.

26 Bertucci F, Viens P, Delpero JR *et al*: High-dose melphalan-based chemotherapy and autologous stem cell transplantation after second look laparotomy in patients with chemosensitive advanced ovarian carcinoma: long-term results. Bone Marrow Transplant *26(1)*: 61-67, 2000.

27 Zambelli A, Montagna D, Da Prada GA *et al*: Evaluation of infectious complications and immune recovery following high-dose chemotherapy (HDC) and autologous peripheral blood progenitor cell transplantation (PBPC-T) in 148 breast cancer patients. Anticancer Res *22(6B)*: 3701-3708, 2002.

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